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HELMINTHOSPORIUM LEAF SPOT OF SUGAR CANE IN PORTO RICO

(Preliminary Paper)

MELVILLE T. COOK

Soon after the arrival of the writer in Porto Rico (July 1923) his attention was called to two extremely interesting leaf-spot diseases of the sugar cane. One which was temporarily designated as the "Manatí disease" because it was first found in the vicinity of Manatí, but which has since been found in other localities along the eastern half of the north coast. The other was temporarily designated as the "Santa Rita disease" because it was found on and in the vicinity of the Santa Rita plantation near Guánica. No well marked cases of this disease have been found in other places. A study of these diseases indicated that they are both caused by *Helminthosporium sacchari* Butler or by closely related varieties or species of *Helminthosporium*. These diseases may be described as follows:

MANATÍ DISEASE

This disease starts as very small reddish, occasionally black spots. If red, a black center develops very quickly. The spot becomes very much elongated but usually remains narrow. The center is surrounded by a yellowish zone which may be light green or almost white. These colors grade or blend into each other and vary greatly in relative amounts. Some of the spots remain red until one-fourth inch in length before showing the black center. Any one of the three colors may predominate. When the spots grow old, they usually develop ashy colored centers. They vary greatly in length from one-four inch to 3 inches or more. Occasionally they form reddish or dark reddish stripes extending from base to tip of leaf but these are probably the results of the unions of two or more spots. In the young spots the colors are usually bright and clear but as the spots grow old the colors become dull

and gradually disappear with the dying of the leaf. The spots may appear on any part of the leaf from midrib to margin but do not occur on the midrib. They are much less severe on the sheath than on the blade. In severe cases the entire leaf with exception of the midrib is practically covered with these spots. The result is the death of the infected parts of the leaves and a checking of the growth of the plant. This disease is most severe during or immediately following periods of heavy rainfall and in extremely severe cases the crop looks brown and almost dead. The disease is most severe on D–109 but what appears to be the same thing occurs in a much less severe form on B–3412, D–117, D–433, FC–214, FC–306, PR–260, PR–412, PR–430, PR–561, SC–12(4).

The sporophores are in clusters of from four to twenty, unbranched, 3–10 septate, dark green to brown or black, only slightly geniculate, 25 to 115 × 5 microns, spore slightly curved, 5 to 11 septate, 45–110 × 12 microns. (Figures 1 and 2.)

SANTA RITA DISEASE

This disease starts with minute reddish spots. As they increase in size they may occasionally assume the same characters as those of the Manatí disease but usually are wider, blunt with very pronounced red color which gradually becomes more or less purple. In more advanced stages the spots are larger and irregular in shape. This irregularity is apparently the result of the union of both old and young spots. The result is that the spots become very large and irregular in shape and sometimes include small spots of green, apparently healthy tissues. They may now be more appropriately called blotches. The color varies from red to dark purple, the latter color predominating. The surrounding tissue is usually pale yellow. The amount of purple blotch increases until it is far in excess of the green on the lower half of the leaf. The upper or outer half of the leaf shows very little or no spotting but with the advancement of the disease on the lower half, it becomes yellow and ashy brown. The sheath is finally attacked but not until the disease is well advanced on the blade. The result is a checking of the growth of the cane. This disease is very severe. Severe forms of the disease have not been found in any other place than at Santa Rita nor on any other variety than B. H. 10(12). However, milder forms of the disease have been found on other varieties in that vicinity.

The sporophores are in clusters of from 3 to 6, unbranched,

6–10 septate, dark green to brown or black, straight or geniculate, bearing a single spore at each bend, 60–300 × 12–14 microns, spores slightly curved, 4–10 septate, 30–95 × 12–15 microns. (Figs. 4 and 5.)

The characters of the fungus in both cases are those of the genus *Helminthosporium*. There was a severe outbreak of the "Manatí disease" during the early part of the summer of 1923, but at the time of the arrival of the writer, it was rather inconspicuous. However, the Santa Rita disease was very prominent. The first studies revealed such a small number of spores as to be very unsatisfactory. Later, it was found that if leaves on which the diseases were well advanced were collected and kept in a moist chamber from 24 to 48 hours spores would be produced in very great abundance. However, they were easily detached and it was not always easy to find them in abundance.

Helminthosporium sacchari Butler has been reported from various parts of the Island by Johnston and Stevenson. This species was described by Butler from India in 1913 [1] as follows:

"The infected leaves first show small red spots, which spread rapidly, chiefly in a longitudinal direction and, especially toward the tip of the leaf, may run together to form long streaks. The centre of the spot soon changes to a dirty straw color, around which the margin remains red for a time and then changes to dark brown. The spots occur equally on the midrib, where they may be confused with those caused by the leaf form of *Colletotrichum faleatum* and on the thinner part of the leaf. When numerous, they cause death of the leaf tissues beyond the limits of the spots; the tip of the leaf often withers completely and there may be long withered strips down the margins.

"The sporophores are stout, erect, rather rigid hyphae, which arise from the peripheral cells of the stromata. They are usually unbranched, 3 to 10 septate, dark greenish-brown below, paler above and several times bent or 'geniculate'. Spores are produced at each bend and at the apex, the lowest being the first formed and the bent condition being due to the spores being always apical at first and being then pushed to one side by continued growth of the sporophore from just below the insertion of the spore. The sporophores are 100 to 190 microns long, by 5.5 to 7.5 microns broad.

"The spores are borne singly and readily fall off. They are cylindrical or long elliptical in shape, with very thick walls, and divided into from 4 to 11 compartments by broad thick partitions. The color varies from olive green to brown and the size from 35 to 60 microns long, by 8.5 to 12 microns broad.

"*Helminthosporium Sacchari* Butl. n. sp. Maculis amphigenis, elongatis, initio rubris, dein avellaneis, vel straminais ac ferrugineo-marginatis, 3–25 × 2–6 mm.; caestiputulis minutis, atris; hyphis fertilibus erectis, simplicibus, 3–10

[1] Butler, E. J., and Kahn, A. H. Some new Sugar-Cane Diseases. Memoirs of the Department of Agriculture in India (Botanical Series) Vol. VI, No. 6.

[2] Butler, E. J., and A. Hafix Kahn. Red Rot of Sugar Cane. Memoirs of the Department of Agriculture in India, Bot. Ser., Vol. VI, No. 5, 1913.

septatis, geniculatis, olivaceo-brunneos, apice pallidioribus, 100–190 × 5.5–7.5 microns; conidiis amrogenis, cylindriaceis vel oblongo-ellipticis, utrinque rotundatis, 3–10 septatis, crassissime tunicatis, olivaceo-brunneis, 35–60 × 8.5–12 microns.''

Butler's description of the spot is very brief and unsatisfactory but a comparison of the measurements of the sporophores and spores as given by Butler with the two forms in Porto Rico shows that the sporophores of the Manatí fungus are smaller than those of *H. sacchari* while those of the Santa Rita fungus are larger. The spores of both the Porto Rican forms tend to run somewhat larger than the spores of *H. sacchari.* The writer judging from both spots and causal organism believes that the Manatí form is more nearly like *H. sacchari.*

J. Van Breda de Haan [1] described a *Cercospora sacchari* from Java producing an ''eye-spot'' disease, as follows:

''Hab. in foliis, quae maculatur, Sacchari officinarum. Hyphae pluriseptate, brunnae, 120–60; conidia 60–80 × 9–12; vernicularia 5–8 septate brunae.''

The complete literature on this disease is not available for the writer. However, Cobb [2] gives a colored plate of this disease which is strikingly similar to the Manatí disease; but spores figured by Cobb are evidently those of *Helminthosporium.* Butler in commenting on the above facts says that ''it appears probably that this fungus is really a *Helminthosporium.*''

Johnston and Stevenson [3] made *C. sacchari* a synonym of *H. sacchari* and described it as follows:

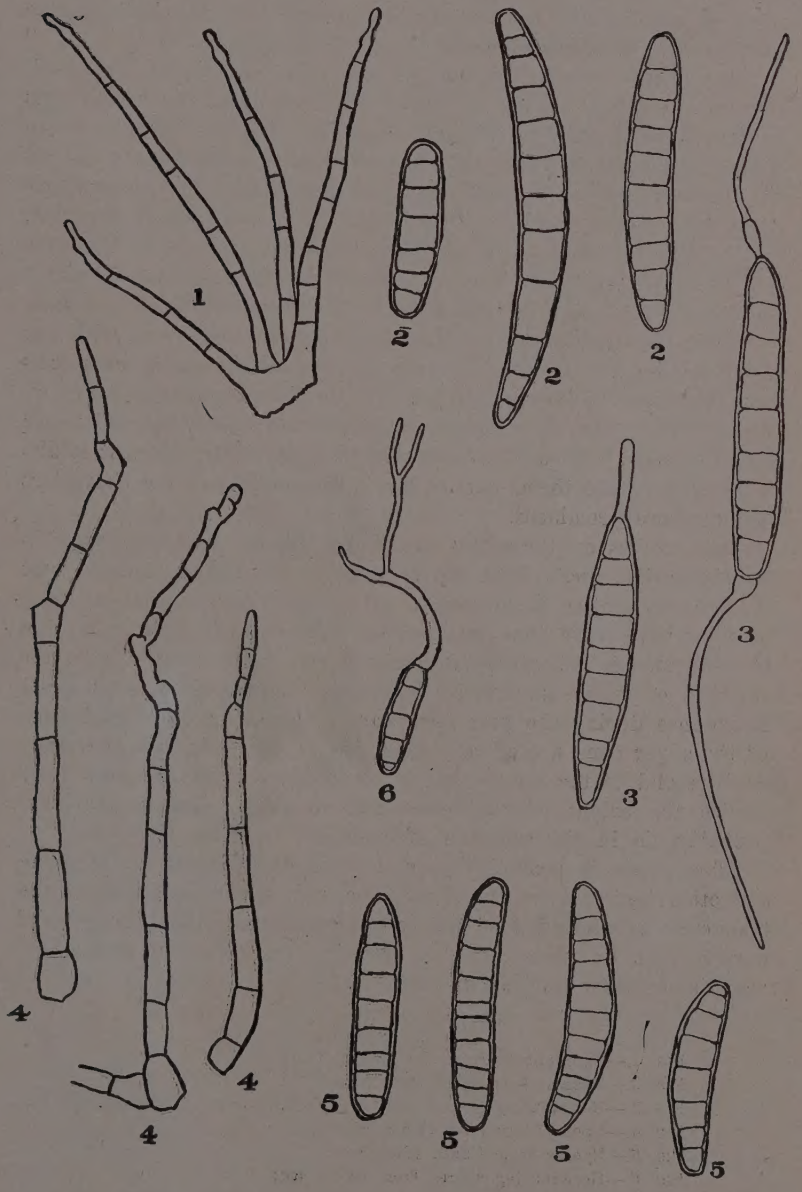
''Hyphae dark, cobwebby, arising from the center of an elongated brown spot on the leaf blade; sporophores more or less erect with single terminal spores; spores several septate with very thick walls, rounded at both ends, 32–90 × 9–14 microns, on conidiophores 120–160 mm. long.''

The measurements given by them are more nearly like those of the Manatí than of the Santa Rita form. Judging from the studies up to this time, it appears (1) that *H. sacchari* in Porto Rico is subject to considerable variations which may be due to local conditions or to varieties of host plants or to unknown causes; (2) that the Manatí form is the same or a closely related species and the Santa Rita form is a variety or possibly a new species. How-

[1] Breda de Haan, J. Van. Root Rot en anders Ziekten en het Zuikerriet, Meded. van het Proefstation, West Java, XVI, 1892.

[2] Cobb, N. A. Fungus Maladies of the Sugar Cane. Report of work of the Experiment Station of the Hawaiian Sugar Planter's Association. Bul. 6, 1909. (Division of Pathology and Physiology, Plate IV.)

[3] Johnston, John R., and Stevenson, John A. Sugar-Cane Fungi and Diseases of Porto Rico. Journal of the Dept. of Agriculture of P. R., 1: 177–251 (1917). (See page 203.)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ever, it may be that the "Santa Rita disease" as described may be due in part to other causes.

The spores of both the Porto Rican forms germinate very readily in water, and so far as observed always from the apical cells (Figures No. 3 and 6). When a suspension of spores in water is applied to the young leaves or when pieces of diseased leaves are set in the axils of the young leaves and kept moist the diseases are transmitted very readily. It is important to keep the plants wet. The young leaves contract the disease much more readily than the old ones. Minute spots may be seen by careful examination within 36 hours after infection. Spots are very distinct within 3 or 4 days.

Thus far inoculation experiments have been carried on with but two varieties, the D–109 on which the Manatí disease is most common and most severe and the B. H. 10 (12), the only variety on which the Santa Rita disease is important. Both varieties are easily infected with either fungus and in the young stages it is difficult to separate them, but as they advance in age the characters become more prominent.

Our studies on these two prominent diseases and also on *Helminthosporium* spots from other parts of the Island indicate that *H. sacchari* Butler is subject to great variations or that we may possibly have more than one species. The variations may be due to variations in climate in different parts of the Island or to the varieties of sugar cane which have been developed in such great abundance during the past few years. These and other leaf spots of the sugar cane are of very great importance. In fact they may rank second to the mosaic but it is doubtful if the growers fully realize the extent of the losses due to them. The control will probably lie in the selection of resistant varieties.

This paper is preliminary to a more extensive study of these and other leaf spots of the sugar cane with special reference to the taxonomic relationships of the causal organisms; the influences of environment on them and the relative resistance of sugar-cane varieties to these various diseases.

EXPLANATION OF PLATE

FIG. 1.—Sporophores from the Manatí form.
FIG. 2.—Spores from the Manatí form.
FIG. 3.—Germinating spores from the Manatí form.
FIG. 4.—Sporophores from Santa Rita form.
FIG. 5.—Spores from Santa Rita form.
FIG. 6.—Germinating spores from Santa Rita form.

A METHOD IN MICRO-TECHNIQUE

By Melville T. Cook

It has long been recognized that in the preparation of many kinds of pathological plant tissue for microscopical study, the causal bacteria or the spores of the causal fungus are usually lost in the fixing, dehydrating or other processes. Therefore, the preparation usually shows nothing but the tissues of the host and some of the fungus mycelium. The writer presents a method which may be of value to his fellow workers. By this method it is possible to retain bacteria, spores of *Colletotrichum*, *Gloeosporium*, the rusts and many other fungi in position.

The method consists in covering the surface with a very thin layer of agar made up at the rate of 15 or 20 grams to the 1,000 cc. of water. The agar should be heated to the melting point, poured over the surface of the material, then drained off and allowed to harden. Cut the material into pieces of the proper size and drop into the fixing fluid.

In the use of this method the following points must be taken into consideration:

(1) A hard agar is more satisfactory than a soft agar.

(2) Allow the agar to harden thoroughly before cutting.

(3) A thin layer is more satisfactory than a thick layer. A thick layer frequently separates from the material, carrying some of the spores with it.

(4) Cut with a sharp knife, so as not to break the film of agar from the surface of the material.

(5) There must always be one or more freshly cut surfaces to permit the entrance of the killing fluid. These fluids do not penetrate the agar.

(6) The pieces of material should be small, so as to permit quick penetration.

(7) The heating of the paraffine will not melt the agar.

(8) Cultures grown in petri dishes may be killed and fixed by this method by pouring a thin layer of agar on the surface. Very small pieces should be used for fixing.

The writer has used this method for some time with excellent results in the study of plant tissues infected with bacteria, and a number of parasitic fungi. The method has not been found satisfactory with *Cercospora* and only fairly satisfactory with *Septoria*.

COCONUT FALL

(Preliminary Paper)

MELVILLE T. COOK

In December of 1923 the attention of the writer was called to the falling of nuts and leaves from coconut palms. The section in which this disease was discovered contained a very large number of trees and in some places the majority of them were infected.

The symptoms were: (1) A drooping of the lower leaves which was due to a black decay at the base of the petioles. These leaves drop prematurely.

(2) A premature dropping of the nuts in various stages of growth from the very smallest up to those that were practically mature. All these nuts showed a black discoloration at the base. In some cases this blackened area became dry after the dropping of the nuts, while in other cases it developed into a soft rot. This variation was undoubtedly due to the dry or wet condition. In case the disease did not attack the nuts until they were practically mature, the husk only was infected and there was no loss. In those cases in which the young nuts were infected, they fell before maturity and the losses were very heavy.

(3) The infection was less on the tall than on the low trees.

This disease was never observed to kill a tree but loss of nuts was alarming and the drooping of the lower leaves was very unsightly.

An examination of the infected areas showed an exceptionally large amount of litter consisting of old leaves, husks and nuts in various stages of decay.

A study of the diseased nuts showed a very general infection with *Thielaviopsis paradoxa* (De Syner v. Hohn). In many cases pure cultures were secured direct. A study of the litter showed a very general and very heavy infection with this same fungus.

A few months later our attention was called to two isolated trees which were almost ready to bear fruit. These trees were not making a satisfactory growth; the new leaves were not fully expanded and were very much crumpled. These trees were cut and examined. In the first one a very large amount of decay was found in the center of the trunk but none in any other part. The cultures were overrun with saprophytes and very unsatisfactory for study. In

the second there was a small streak of decay from the top to the base of the tree. Cultures from this decay gave abundant growth of *T. paradoxa* but it was impossible to determine whether the infection was primary or secondary. This fungus was reported in the 1912 Annual Report of the Porto Rico Agricultural Experiment Station at Mayagüez as attacking the leaves of the coconut palm but no mention was made of its attacking the nuts. It has also been reported from Jamaica as the cause of a disease of the coconut known as "leaf-bitten," but the symptoms are entirely different from those of the disease found here. It has also been reported from Ceylon (Cries. and Agric. Journ. Royal Botanical Garden, Ceylon. 4 [1909] No. 22 S. Sundararaman on "The Coconut-Bleeding Disease." Bul. 127. Agricultural Research Institute, Pusa) as causing bleeding and decay of the trunks of the coconut palm, but no such condition here has come to our attention. It has also been reported from Florida as the cause of a trunk decay (H. R. Fulton. Phytopothology 12: 398–399, 1922). This fungus is also the cause of a root and stem rot of sugar cane and a fruit rot of the pineapple. In some parts of the world it reduces the germination of the sugar cane. It not only causes a rotting of pineapple fruits but frequently attacks and kills the slips after setting.

Inoculation from pure cultures of this fungus was made on trees in the Station grounds where there was no evidence of the disease. These inoculations were made by drenching the inflorescence and young nuts with water to make conditions as nearly as possible like those following rainfall. Spore of the fungus from pure culture were mixed in water in an ordinary atomizer and sprayed on the inflorescence and young nuts. This was done about 4 o'clock in the afternoon. In a few days we had a very general infection and falling of nuts from the smallest up to those about one-fourth grown. The symptoms were typical and the organism was very easily recovered from these nuts. No effort was made to disinfect these trees, but the disease disappeared in a very short time indicating that it was controlled largely by natural conditions.

The moist litter in the plantations furnishes ideal conditions for the growing of this fungus and the spores are produced in great abundance and readily carried by wind currents. The greater infection on the low rather than on the tall trees indicates that the greatest source of infection probably comes from the litter on the ground and that the spores of the fungus are carried up by wind currents. However, the reduced infection in the tall trees may be

partly due to the drying influence of the sun which would naturally make conditions unfavorable for infection.

An examination of several groves showed that the disease was most prevalent in the presence of moisture and litter. The disease was not found in the interior and in only a few places along the coast.

We did not carry on any work for the control of this disease but recommended a very general cleaning up of all litter in the plantations, and a removal of the diseased leaves and nuts so far as possible. This treatment was carried out and in some places the ground has been plowed. The reports indicate that the disease has practically disappeared.

The writer was assisted in his work by Mr. R. A. Toro and by Mr. J. A. B. Nolla.

A BACTERIAL WILT OF COSMOS

(Preliminary Paper)

MELVILLE T. COOK

This disease appears in the Cosmos plants of any age and has proved very destructive at the Insular Experiment Station and in flower gardens in this vicinity. The foliage wilts and finally turns black. If the bark is removed from the stem just above the surface of the ground, the tissues between the bark and the wood are found to be brown or black. These discolorations may extend to a considerable distance into the branches and the roots. A microscopic examination of a cross section of the discolored part shows an abundance of bacteria and sometimes a fungus in the tracheary tubes. Cultures almost invariably developed a bacterium and a fusarium. Inoculation with these two organisms proved that the bacteria was the cause of the disease.

On potato agar the organism starts slowly but produces a heavy white or almost clear growth within two days.

The growth on oat-meal agar is less than on potato.

The growth on Cooks No. II agar is heavier than on the potato and is slightly tinted yellow. There is considerable gas formation on the third day.

A BACTERIAL WILT OF EGGPLANTS

(Preliminary Paper)

MELVILLE T. COOK

A wilting of the eggplants at the Insular Experiment Station is the cause of heavy losses. Specimens of this disease have been sent to us from other parts of the Island.

The disease does not attack the plants until they begin blooming and fruiting but, when a plant is attacked the disease progresses very rapidly. The first evidence of the disease is a wilting and drying of the lower leaves, followed by a gradual wilting of all the leaves, those at the top wilting last. The leaves are often dry before they have lost all their green color. They finally become brown. If the bark on the stem is removed at the surface of the ground a black zone is found between the bark and the hard wood. This blackening of the tissues can be traced for a considerable distance into the roots and branches, especially in the severe cases. A microscopic examination of a cross section of this blackened tissue shows that the tracheary tubes are filled with bacteria.

The bacteria are very easily isolated and grown in culture. In fact, most of the cultures are pure. This organism was inoculated into plants of various ages by means of punctures into the stem just below the surface of the ground. These punctures were covered by a pad of cotton and proper checks made. Regardless of the ages of the plants inoculated, there was no evidence of the disease in any of them until they commenced blooming or bearing fruit, when they developed the symptoms previously described. Tomato, pepper and tobacco plants were inoculated with this organism and grown along side the inoculated eggplants, but did not develop the disease.

This disease occurs on every crop planted in our truck crop plots but not in crops planted in soil not previously used for eggplants, which indicates that the organism persists in the soil.

The growth on potato agar was heavy, white, slightly tinted with yellow on the second day with a tendency to liquify the agar and form a gas.

The growth on Cooks No. II was almost equally good, white, tinted with yellow and tendency to form gas.

The growth on oatmeal was slight and tinted with yellow.

Diseased and Healthy Eggplants